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EXPERIMENTAL PRODUCTION OF BOVINE MASTITIS WITH STREPTOCOCCI AND OTHER BACTERIA

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Certain aspects of the etiology of bovine mastitis have been studied but the majority of workers have dealt with organisms from milk from infected udders. The bacteria isolated have fallen, for the most part, into two groups, hemolytic and nonhemolytic streptococci. A smaller percentage is due to the staphylococci, *B. pyogenes* and members of the colon-aerogenes group. In the literature are few references to the experimental production of mastitis. This is due, in all probability, to the fact that the experiment is an expensive procedure because one cannot carry it on without a possibility of damaging the value of the animal.

Franck¹ produced mastitis by introducing infected fluids into the teat canal then injecting the milk from the diseased udder into that of a normal animal. Guillebeau and Hess² state that they have injected subcutaneously into goats an organism which they call *B. coli*, isolated from a case of mastitis, the result being localization of the organism in the mammary gland of the goat followed by a severe inflammation of the udder. Kitt³ states that he has produced mastitis in cattle by immersing the end of the teat in a fluid culture of colon bacilli. Jensen⁴ and Fauss⁵ produced an acute mastitis with *B. coli* and other bacteria. Bang⁶ and Wall⁷ caused mastitis in cattle with streptococci, staphylococci, colon bacilli and *Bacillus pyogenes*. Bang introduced the various organisms into the udder by means of a glass rod dipped in the culture. Meyer⁸ infected 2 animals with streptococci. He states that the inflammation is caused by the metabolic products of the organism and not by the streptococcus itself. Harrison and Cumming⁹ smeared the ends of the teats of the cows with *B. prodigiosus*, *B. fluorescens liquefaciens* and an organism they called *Bact. exiguum* which is a chromogenic bacterium found occasionally in water and milk. They found comparatively small numbers of the organisms in the fore milk for the first 2 or 3 milkings. They did not recognize any inflammation of the udder. Gminder¹⁰ produced mastitis in goats by injecting streptococci

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¹ Deutsch. Ztschr. f. Tiermed., 1876, 2, p. 456.

² Kolle and Von Wassermann's Handbuch der path. Mikroorganismen, 1913, 6, p. 96.

³ Lehrbuch der pathologischen Anatomie der Haustiere, 1900, 1, p. 200.

⁴ Lubarsch and Ostertag's Ergebnisse der allgem. Pathologie, 1897, 4, p. 830.

⁵ Berner Diss., 1908.

⁶ Tidskrift för Veterinärer, 1888, 18, p. 226.

⁷ Die Enterenzündungen der Kuh, 1908.

⁸ Arch. Wiss. u. Prakt. Tierheilk., 1910, 36, p. 583.

⁹ Jour. Applied Microscopy, 1902, 5, p. 2086.

isolated from chronic cases in cattle. Savage¹¹ failed to produce mastitis in goats with streptococci from tonsillitis and other diseases of man, with the exception of 2 cases in which he observed a slight inflammation of the udder. He introduced the material into the teat canal with a platinum needle. In connection with an outbreak of septic sore throat, which was traced to an infected milk supply, Davis and Capps¹² smeared the uninjured surface of the teats of a cow 3 successive days with cultures of hemolytic streptococci isolated from cases of tonsillitis. Two strains were used in the experiment. The results were negative. Later they made a similar experiment, after an abrasion had been made near the meatus of the teat canal, with positive results. They also introduced well up into the teat canal, by means of a catheter, 8 c c of a 24-hour milk culture of a hemolytic streptococcus, producing a typical mastitis. Mathers¹³ produced mastitis in cattle with virulent hemolytic streptococci from bovine mastitis and from a fatal case of streptococcic peritonitis in man. In neither case did the quarter recover. He produced acute inflammation of the udder with a nonvirulent, nonhemolytic *Streptococcus lacticus* from which infection the quarter returned to normal in a short time. A condition similar to that produced by *Streptococcus lacticus* was brought about by a culture of a nonvirulent hemolytic streptococcus such as is found in normal milk. From 1 to 5 c c of the living culture was injected directly into the teat canal in the different experiments.

Most of the earlier workers with the exception of those mentioned used 10 and 12 c c of a broth culture of the different organisms and injected the material into the canal.

My work was undertaken to ascertain the ability of certain bacteria to produce mastitis in cattle. These organisms, with the exception of *Bact. pyocyaneum* and *Pasteurella bovisepctica*, were isolated from cases of mastitis and from diseased genital organs of cattle. I was particularly interested in the question of immunity to these diseases of the udder, whether there is immunity after recovery from this condition, and whether immunity can be established by injecting the organism into the tissues of the body other than the udder.

It is common knowledge that cows put on test for production of milk and butterfat are susceptible to attacks of mastitis, especially when large amounts of food with a high protein content are fed to the animals, but the source of the infection is still unsatisfactorily explained. While doing some work with calves in a large purebred herd, I became keenly interested in this condition, but the value of the animal would not permit any experimental studies. Bacteriologic examinations from four cases showed that the milk contained a nonhemolytic streptococcus and large numbers of leukocytes. As this condition had occurred before in the herd, every precaution was taken by the tester and herdsman to eliminate any external source of infection. The tester disinfected his

¹⁰ *Centralbl. f. Bakteriol.*, 1912, 63, p. 152.

¹¹ *Rep. of Medical Officer, Local Gov't. Board of London*, 1906, '07, '08, '09.

¹² *Jour. Infect. Dis.*, 1914, 15, p. 135.

¹³ *Ibid.*, 1916, 19, p. 222.

hands before milking each animal, and the teats were immersed in an antiseptic before and after each milking. Other sanitary conditions were unusually good, necessarily so because the milk was being supplied for a city.

Thirteen animals ranging in age from 2½ to 11 years, and with apparently normal udders, were used for this work. A bacteriologic examination was made of the milk from the 4 quarters of each udder before the injection of the different organisms. The milk was collected in 4-ounce sterile containers after the ends of the teats had been disinfected with 2% phenol solution and the fore milk discarded. The medium used in the work was a veal infusion agar to which was added sterile horse serum or corpuscles. One-tenth of a c c of milk from the samples was cultivated after shaking thoroughly. Smears also were made from the samples with milk and stained with methylene blue. In 4 of the cows no bacteria were recovered from the milk. In the other cases a few colonies of micrococci producing different pigments, chiefly of the albus and aureus varieties, were found. No streptococci were cultivated from the milk. Occasionally a cell or organism was observed in the smears, but this was not considered as signifying that inflammation existed in the udder.

With the exception of the specific cases mentioned, all cultures used for injection were 24-hour broth cultures. The material was injected into the milk duct by means of a small teat cannula fitted to a 10 c c record syringe. The tip of the cannula was inserted just past the sphincter muscle at the end of the teat and injected at this place. The cultures were injected immediately after the animal had been milked, which was about 4 p. m. The animals were milked by one man with occasional assistance from me. The first personal observation was made about 22 hours after the injection, when the samples were collected, and these were examined and new samples collected once every day thereafter. The report of the condition of the udder and of the animal at the first milking after the injection, which was at 4 a. m., was given to me by the herdsman.

Samples of mastitis milk containing nonhemolytic streptococci were injected into one quarter of the udder of each of 7 animals. Three samples of milk, designated as A, B and C, were obtained from 3 different herds in which mastitis existed in varying degrees. One heifer received 5 c c of sample A 15 minutes before the animal was milked dry. Three older cows, one not in milk (R34), received injections of 5 c c of this same sample A. The aged cow (R34) mentioned in the foregoing, which was dry, received in another quarter 1 c c of sample B. A heifer also received 0.5 c c of this sample.

Sample A proved to be very virulent. Two of the animals suffered from a general reaction and rise in temperature which was followed by a severe diarrhea for 2 days. The third animal did not show the general reaction, but the condition of udder and milk was similar to that of the other 2. The quarter became greatly enlarged, hot and painful, and the material obtained from it in each case was composed of yellowish, stringy pus. The quarters finally atrophied, but they still gave a little pus from which the nonhemolytic streptococcus could be isolated for 4 months, at which time the animals were slaughtered.

The dry cow did not show signs of a reaction from the infection of sample A. The organism was isolated once from the quarter 24 hours after the injection and a few leukocytes were present in the smears, but these appeared greatly disintegrated and were probably the cells that were in the sample that was injected.

The heifer that received 0.5 c.c. of sample B gave a similar general and local reaction to the injection as did the cows mentioned in the foregoing. The quarter atrophied and leukocytes and streptococci were isolated until the time of slaughter. R 34 showed a few similar cells in the secretion obtained from the udder, but the quarter showed no evidence of inflammation. This condition disappeared in a few days.

A quarter of R 51 was injected with 1 c.c. of a 24-hour broth culture of a nonhemolytic streptococcus isolated from the milk sample C. This produced a severe general reaction. The quarter enlarged and became hot and painful. The milk changed to a thick, stringy, yellowish pus. Later the quarters atrophied, and the condition continued the same for a month when the animal was slaughtered. There were large numbers of cells and streptococci present at this time.

Three older animals were also injected with 1 c.c. of a nonhemolytic streptococcus from milk sample B. The cultures of this strain did not prove to be very virulent to these animals. In one case there was no reaction after the injection. The other 2 cases revealed a little pus in the milk, but the udders remained normal. The leukocytes cleared up 8 days after injection, and the streptococcus was not found after the sixth day.

Samples of milk containing hemolytic streptococci were obtained from 3 herds. These are designated as samples 1, 2 and 3. Two aged cows giving milk and R 34 not in milk (the same animal that was used before) were injected with 3 c.c. of sample 1. One animal received 1 c.c. of sample 2 and one animal received 1 c.c. of sample 3. R 34 showed no evidence of infection after the injection of sample 1. The hemolytic streptococcus was recovered from the 24 and 48 hour samples. No polymorphonuclear leukocytes were found in the smears. One animal had a general reaction and the quarter became enlarged, hot and tender. All that could be obtained from the quarter was a small amount of pus that contained large numbers of the organism. This resulted in the atrophy of the quarter, but the milk returned to normal 25 days after injection. The third animal that received the injection from sample 1 was nearly dry, and the quarter apparently was not enlarged or inflamed. The milk showed a few cells and also the organism for 3 days. The quarter and milk were normal on the fifth day after the injection.

Four cultures of a hemolytic streptococcus isolated from samples 1, 2, 3 and from a fourth sample were used on several animals. One quarter of the udder of each of 3 animals, one aged cow and 2 heifers, received 1 c.c. of a 24-hour broth culture of sample 1. The aged cow apparently suffered no ill effects from

the injection. The quarters of the heifers showed a slight swelling and a few flocculi of pus in the milk. One animal was slaughtered 10 days after the injection, and she still showed a few cells and a large number of streptococci at that time. The cells and bacteria in the second heifer disappeared 6 days after she received the injection.

Injections of cultures from milk sample 2 were made on 2 cows and 2 heifers, one of which had progressed about 6 months in her first pregnancy and had never given milk. This heifer and one of the cows failed to show a reaction after receiving the material, but the culture was isolated from this cow for 3 milkings afterward. One heifer and one cow showed a slight inflammation of the udders and a few flocculi of pus in the milk. In these 2 animals the condition remained the same until they were slaughtered.

A culture from sample 3 was injected into one heifer. This produced a slight swelling of the udder and a little pus in the milk. The condition continued for one month at which time the animal was slaughtered.

Three cc of a 24-hour broth culture from sample 4 was injected into one animal that had suffered from mastitis due to a natural infection, the cause of which was not determined. This produced a slight inflammation of the udder and a few leukocytes in the milk, but both conditions cleared up in 3 days. The injection was repeated 6 days later with negative results.

A quarter from the udder of each of eight different animals was injected with *Streptococcus viridans*. Three different cultures of this organism which had been isolated from diseased ovaries and uteri of cattle were used.

Each of 3 aged animals received 6 cc of one culture. One of the cows was not in milk. Three 3-year old heifers were given 2 cc of the second culture and one 5-year old cow and another 3-year old heifer received 2 cc of the third culture.

The results of these injections were quite similar in all cases. As a rule, the inflammation was at its height in 24 hours. Three animals showed no clinical evidence of any inflammation. In 2 other cases, the samples showed no bacteria or leukocytes. In the remaining animals there was a slight but variable manifestation of an inflammation of the quarter. The quarters were rather firm to the touch and somewhat enlarged. Usually the observable inflammation subsided in 2 days, and the samples of milk showed no cells or streptococci 7 days later, with the exception of the milk of the dry cow. The small amount of milk in her udder thickened until it was practically nothing but strings of pus. It was 22 days before the cells and bacteria cleared up.

In 3 animals 2 cc of a fourth culture were injected into another quarter 20 days later. In these cases there was a slight external inflammation, and the milk showed a few flocculi composed chiefly of polymorphonuclear leukocytes and streptococci in short chains of 4 and 6 cocci. This condition cleared up in 4 days.

A culture of a nonhemolytic *Staphylococcus aureus* was isolated from a case of mastitis. A quarter of the udder of each of 3 heifers and 2 older animals was injected with 2 cc of a 24-hour broth culture of the organism. One of the older animals, R7, was dry. The contents of her quarter became thick and the staphylococcus was recovered from the quarter 6 days after the injection. Leukocytes were present for 10 days. The udder showed no external evidence of inflammation. The other 4 animals suffered a severe general reaction. There was a marked rise in temperature, loss of appetite, stamping of feet, etc. These symptoms were evident 8 hours after injection and lasted 36 hours. The

quarters became greatly enlarged, hot and painful. The secretion from the glands was thick, viscid, fetid pus. R70 died 10 days later from a septicemia resulting from the infected quarter. The organism in question was isolated from all the tissues of the body of the animal. In the case of one animal, R46, the inflammation spread from the injected quarter to the other 3 quarters. These 3 quarters were not enlarged, but the milk contained a large number of leukocytes and nonhemolytic *Staphylococcus aureus*. The 3 adjacent quarters cleaned up in 6 days. This same animal was injected in a second quarter one month later. The animal did not show any general reaction, but the quarter was destroyed as in the first case.

Abscesses finally formed in the infected quarters of the other 3 animals. The quarters atrophied and *Staphylococcus aureus* was recovered from them until the time of slaughter 3 months later.

Two c.c. of a 24-hour broth culture of a 3 sugar colon isolated from the vagina of a cow was injected into a quarter of the udder of each of 3 animals. One was 10 years old, one 6 years, and the third, 3 years. In 5 hours the quarters were greatly enlarged, hot and painful. The milk was not altered in character, but there was a large number of organisms present. In 12 hours, nothing but strings of yellow viscid pus could be drawn from the quarter. Twenty-four hours later, the condition was about the same, but 48 hours after the injection there was marked improvement. The acute inflammation had subsided, and the milk from the quarter was approaching normal. There was still a larger number of leukocytes and bacteria present. In 6 days the bacteria had disappeared from the smears and in 8 days no cells or bacteria were present, with the exception of one case which showed a few cells but no bacteria. Ten days after the first injection the same amount of the same culture was reinjected into these quarters which had just recovered from the first injection. The same changes took place as the first time, but the quarters did not swell so badly and the milk did not contain so much pus. These conditions cleared up 3 days sooner than those resulting from the first injection of the colon organism.

The quarters of 2 animals, one heifer and one cow, were injected with 5 c.c. of a 24-hour broth culture of *Bacterium abortum*. The tissues of the quarters did not show any evidence of inflammation. In 24 hours the milk contained small clumps of cells that were visible when the milk was squirted on the palm of the hand. The organism was recovered in culture 3 days after injection. Apparently the organism did not establish itself in the udder of the animal because guinea-pig inoculations after the third day were also negative.

The quarter of one heifer was injected with 1 c.c. of a 24-hour broth culture of *Bact. pyocyaneum*. In 24 hours there was a slight enlargement of the quarter, and the milk contained dark yellow shreds of pus. The organism was recovered from milk 4 days after injection, but the cells did not clear up until the tenth day.

A quarter of the udder of each of 2 heifers and of one older cow was injected with 1 c.c. of a 24-hour broth culture of *Pasteurella bovisepctica* isolated from the pneumonic lung of a calf. This caused a severe general reaction in all 3 animals. In 20 hours the temperature was elevated to 104.6, 104.2, and 103.9 F. The animals were dull, would not eat, and apparently suffered much pain. They were very uneasy, and would lie down and get up immediately. The quarters were hard and firm and bluish in color. Only a little reddish serum with a few cells could be obtained from them. The milk supply was markedly

decreased in the other 3 quarters. Rabbits injected with 0.5 cc of the serum obtained from the affected quarter died in 12 hours. The general symptoms subsided in 36 hours and *Pasteurella bovisepctica* was recovered from the secretion from the quarters 3 and 4 days after the injection. The affected quarters atrophied but abscesses did not form, and they remained in this condition until the animals were slaughtered.

DISCUSSION AND SUMMARY

The results show that some bacteria possess a marked degree of pathogenicity while others possess none, or give only a slight reaction which would probably occur from the introduction of any foreign fluid into the milk ducts or cisterns. One can summarize in general terms only because the resistance of the animal must be taken into consideration, and the intricate phenomenon following the introduction of foreign protein into the animal tissue is difficult to follow. The life histories of almost all of these animals have been accurately kept, and, with one exception, there is no record of the animals having suffered from infected udders up to the time the experiments were begun.

It is apparent that a certain quantity of infected milk containing a nonhemolytic streptococcus causes a more severe mastitis than the same amount of a 24-hour broth culture which contains infinitely more organisms than the milk. Whether this is due to the organism losing its virulence immediately on artificial cultivation, or whether it is due to the metabolic products formed in the milk by the streptococci, as Meyer has stated, cannot be determined definitely at this time. The milk containing the hemolytic streptococci and the cultures of the hemolytic streptococci with one or two exceptions did not cause the severe reactions that were caused by the nonhemolytic streptococci. In some cases a mild chronic infection was established, but there was not the marked difference between the ability of the milk and of the culture to produce mastitis that was observed with the nonhemolytic group.

The culture of the *Streptococcus viridans*, *Bacterium abortum* and *Bacterium pyocyaneum* produced practically the same effect on the different animals. It usually consisted of a slight swelling of the quarter infected with the formation of a few flocculi of pus in the milk, which cleared up in a comparatively short time after the injection.

The result of the injection of the organism from the colon-aerogenes group, as stated, shows that its ability to produce mastitis coincides with the work that Jensen did on organisms from this group isolated from the intestinal tract. These brought about an acute inflammation that disappeared about as suddenly as it was produced.

The nonhemolytic cultures of *Staphylococcus aureus* and *Pasteurella bovisseptica* that were used produced a similar type of mastitis with general symptoms and an acute local condition followed by destruction of the quarter. The only noticeable difference was that the *Staphylococcus aureus* produced abscesses in the quarter while *Pasteurella bovisseptica* did not.

Jones¹⁴ has reported finding hemolytic streptococci in the quarters that did not show an inflamed condition as well as in the diseased quarter. I did not observe this in the animals injected with the group of hemolytic streptococci or with any of the other organisms used except in the case of one animal that received an injection of *Staphylococcus aureus*. In this case not only the organism but also large numbers of leukocytes were found in the other quarters.

In most cases, after it was evident that a mastitis was permanently established, an attempt was made to improve or cure the affected quarters by injecting subcutaneously suspensions of the dead organism that had produced the conditions. This seemed to be of no value. In a number of cases suspensions of the dead organisms were injected over a period of fifteen days in gradually increased amounts. One month later there was injected a live culture of the same organism with which an attempt to immunize the animal had been made. The check animals showed no more inflammation of the udder than those receiving the dead suspensions. Milk from the infected quarters was withdrawn and injected subcutaneously. This seemed to be of no value and in some cases produced large abscesses on the animal.

It was observed that the age of the cow and the amount of milk given had an effect on the degree of mastitis produced by the organism. As a rule the cows not in milk showed no ill effects from the injection, and the same animals a few months later after calving responded to the injection according to the organism used. The heifers were much more susceptible and suffered more severely from the injection of the different bacteria used in the experiment.

CONCLUSIONS

Milk containing hemolytic and nonhemolytic streptococci from infected udders produced a more severe mastitis than 24-hour broth cultures of the same organisms when equal amounts were injected into the teat canals of healthy cows.

¹⁴ Jour. Exper. Med., 1918, 28, p. 253.

Streptococcus viridans isolated from diseased genital organs of cattle, *Bacterium abortum* and *Bacterium pyocyaneum* produced only a slight inflammation of the udder when injected into the teat canals. The mastitis cleared up in 48-72 hours after injection.

B. coli produced an acute mastitis which cleared up eight days after injection.

Staphylococcus aureus and *Pasteurella bovisepctica* produced a severe mastitis which destroyed the functional activity of the gland.

The age of the animal and the amount of milk given by the animal at the time of the injection of the various organisms are two factors which influence the degree of mastitis produced.